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EVALUATION OF DIFFERENT CYTOKININS FOR *IN VITRO* MULTIPLICATION OF BANANA VAR. *ROBUSTA*

SAIFULDEEN AHMED HASAN[1] & S. M. KHASIM[2]

[1]Department of Botany and Microbiology, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur, Andhra Pradesh, India

[2]Department of Horticulture, College of Agriculture and Marshes, Thi-Qar University, Iraq

ABSTRACT

Tissue culture technique in multiplication of banana is very efficient and widely applied. The technology will give high-quality, genetically similar plants, free of diseases and nematodes. Much of the planting material used in commercial plantations, especially smallholder production, comes from mass micropropagation. The method of shoot tip culture has been most widely used in banana. However, use of a typical tissue culture protocol is applicable as the technology is highly dependent on genotypes and a number of local variables. In the present study, banana cultivar Robusta was subjected to different cytokinins amended in MS Basal medium. Cytokinins showed differential effects in the micropropagation of Robusta cultivar. Among the different cytokinins tested, BAP was found to be the best cytokinin at an optimum concentration 5mg/L.

KEYWORDS: Banana Cultivar Robusta, *In Vitro* Multiplication, Cytokinins, BAP

INTRODUCTION

The genus *Musa* L. belongs to the family Musaceae of Monocotyledons. Simmonds and Shepherd (1995) demonstrated that cultivated bananas had developed from only two wild species such as *Musa acuminata* Colla *and M.bulbisiana* Colla. In general, banana is a rich source of carbohydrate and vitamins particularly vitamin B. It is also a good source of potassium, phosphorus, calcium and magnesium. The fruit is easy to digest, free from fat and cholesterol. It helps in reducing risk of heart diseases when used regularly and is recommended for patients suffering from high blood pressure, arthritis, ulcers, gastroenteritis and kidney disorders. Banana leaves are used as healthy and hygienic eating plates (Anonymous, 20130).

Application of plant tissue culture for multiplication of disease-free plantlets began in 1960's and early 1970's. The basic technique was extended to the development of commercial laboratory and nurseries in 1970's in both US and Europe (Boxus *et al*., 1977; Holdgate and Aynsley, 1977;). The cytokinins and auxins are of importance in tissue culture as the later one concerned with root formation, the former mainly responsible for shoot formation and growth of buds (North *et al*., 2012). Al-Amin *et al.* (2009) conducted studies on *in vitro* micropropagation of banana to investigate the effect of different concentrations of BAP and NAA on virus-free plant regeneration of banana cv. Bari. Devendrakumar *et al*. (2013) have studied the effect of different concentrations of BAP on shoot induction and shoot proliferation in Banana cultivar 'Pisang Jayee'. Reddy *et al.* (2014) have studied effects of 6-Benzyl aminopurine oni *In vitro* shoot multiplication of Grand Naine (*Musa*). However, there is lack of information on the quality in banana micropropagation. At present, India

is the largest producer of banana in the world with about 30% of total global production. But, the export market share is a meager 1%. Due to huge demand of banana saplings, there is a constant effort to improve the available tissue culture technology and translate the same in the most economical manner so as to supply the quality saplings to the farming sector. Hence, the present investigation has been carried out to evaluate the effect of different cytokinins on production of quality shoots of banana cultivar Robusta.

MATERIALS AND METHODS

The multiplying cultures of banana Robusta were obtained from the Labland's production division, which were previously developed from sword suckers following the standard procedure. The cultures were incubated in growth room at a temperature of 25±2°C and provided with 12 h photoperiod with light intensity of 2500 lux from cool white fluorescent tubes until transferred to the experimental media. After one week, the cultures were screened for contamination. If contaminated, the jars were discarded. Only clean cultures were retained for analysis of multiplication ratio.

Media Preparation and Sterilization

In the present study, MS basal medium (Murashige and Skoog, 1962) was used for transferring the multiplying cultures of Banana cv. Robusta. The basal medium with different concentrations of BAP and 5 mg/L of other cytokinins such as 2iP, kinetin and zeatin were used for testing *in vitro* multiplication ratio of banana cv. Robusta. The composition and concentrations of cytokinins used in MS basal medium for testing multiplication ratio is given in Table 1.

Table 1: Composition and Concentration of Different Plant Growth Regulators Used In MS Basal Medium for Testing Multiplication Ratio of Banana Robusta

Media Code	PGRs Used	Respective Concentrations (mg/L)
01	BAP	5
02	2ip	5
03	Kinetin	5
04	Zeatin	5
05	BAP	25
C 1	NIL	0
C 2	Labland's standard 1st multiplication medium	
C 3	Labland's standard 2nd multiplication medium	

Analysis of in Vitro Multiplication Ratio in Experimental Media

The multiplying cultures of Robusta cultivar were screened for bacterial contaminants. The clean cultures were then dissected to the suitable size and transferred to four different MS media consisting of different cytokinins with same concentration. Two different standard multiplication media standardized by Labland and one MS basal medium without hormones were used as controls. All cultures were inoculated into the chosen test media for the evaluation of multiplication ratio. The exact outcome of the culture growth was assessed only on 30th day. The response obtained on the 60th day was also recorded to evaluate the subsequent hormonal effect on the further growth of cultures. To each of the medium, two cultures were inoculated and at least 10 bottles were maintained for each medium

combination. Thus, for each trial medium, there were 20 replicates. All cultures from each of the trial multiplication medium were transferred to the corresponding multiplication medium to evaluate the multiplication ratio after four weeks.

The cultures were obtained at 25 C under 12h photoperiod for duration of 4 weeks and transferred to respective media. Multiplication ratio was calculated and the results were tabulated. The entire experiment was repeated thrice at an interval of 4 weeks and the average multiplication ratio was considered for final evaluation.

RESULTS AND DISCUSSIONS

The multiplying cultures of Banana cultivar *Robusta* in media without any hormone did not show any significant multiplication ratio both at 30th and 60th day of culturing whereas in Labland's standard multiplication medium (C2 and C3) cultures were proliferated profusely every three weeks with commercial multiplication ratio of three. The quality of cultures was stable with almost same numbers at 30th and 60th day of culture. All the cultures were superior in comparison to the experimental media of the present study.

MS basal medium supplemented with different concentrations of BAP resulted in varying rates of multiplication of axillary buds. When cultures were transferred to medium containing different cytokinins at a concentration of 5 mg/L and also in media containing 25 mg/L showed varied growth response at 30 and 60 days of culture (Tables 2 and 3).

Table 2: Morphological Response of Multiplying Cultures of Banana Variety Robusta to Different Cytokinins after 30 Days of Culture

Media Code	Media Composition	MR	Growth Response (Mean Value of 20 Replicates)				
			Shoot Length (cm)	Shoot Girth (mm)	Leaf Breadth (cm)	No. of Leaves	No. of Roots
01	BAP (5mg/L)	3	2.5	1.7	2.5	5	3
02	2-iP (5mg/L)	1.6	3.5	1.5	3	6	5
03	Kinetin (5m/L)	2.5	2	1.8	2.4	5	4
04	Zeatin (5mg/L)	2.6	1.5	1.6	1.8	4	2
05	BAP (25mg/L)	3	1.4	1.4	2	2	2
C1	No hormones	1	2	1.2	2.5	2	2
C2	Labland's standard Multi media 1	4	3	1.8	3	4	3
C3	Labland's standard Multi media 2	5	3	2	3	6	5

The multiplying cultures of banana cv. Robusta in media containing 5 mg/L BAP showed multiplication ratio of three at 30th and 60th days of culturing. Shoots were supple and compact with each clump having about 6 to 7 shoots (Figure 10A-1). The cultures had highest multiplication ratio when compared to other cytokinins (2iP, kinetin and zeatin) with moderate roots.

Table 3: Morphological Response of Multiplying Cultures of Banana Variety Robusta to Different Cytokinins after 60 Days of Culture

Media Code	Media Composition	MR	Growth Response (Mean Value of 20 Replicates)				
			Shoot Length(cm)	Shoot Girth (mm)	Leaf Breadth (cm)	No. of Leaves	No. of Roots
01	BAP (5mg/L)	3	2.3	1.8	2.3	6	4
02	2-iP (5mg/L)	1.8	3.2	1.4	3	5	5

03	Kinetin (5m/L)	2.5	2	1.7	2.6	4	3
04	Zeatin (5mg/L)	2.7	1.4	1.8	1.6	4	3
05	BAP (25mg/L)	3	1.5	1.4	2	3	2
C1	No hormones	1	2.2	1.2	2.4	2	2
C2	Labland's standard Multi media 1	5	2.8	2	3	5	5
C3	Labland's standard Multi media 2	5	2.8	2	3	6	5

The multiplying cultures of Banana var. *Robusta* when transferred to media containing 5 mg/L 2-iP, showed decreased multiplication ratio of 1.6 and 1.8 at 30th and 60th days of culturing respectively. The shoot length was about 3.2 to 3.5 cm; shoot girth was about 1.4 to 1.5 cm; leaf breadth was 3 cm with 5 to 6 leaves and 5 roots each. The culture had lowest multiplication ratio in comparison to other cytokinins (BAP, kinetin & zeatin) with more roots. Morphologically all the multiplying cultures were greener in colour with wide leaves and thin shoots (Figure 1-2). All the cultures showed shoot elongation and each multiplying clump gave rise to 1 to 2 shoots. The shoot elongation was highest with profuse roots in the cultures. This result indicates that 2-iP encourages cell elongation rather than multiplication

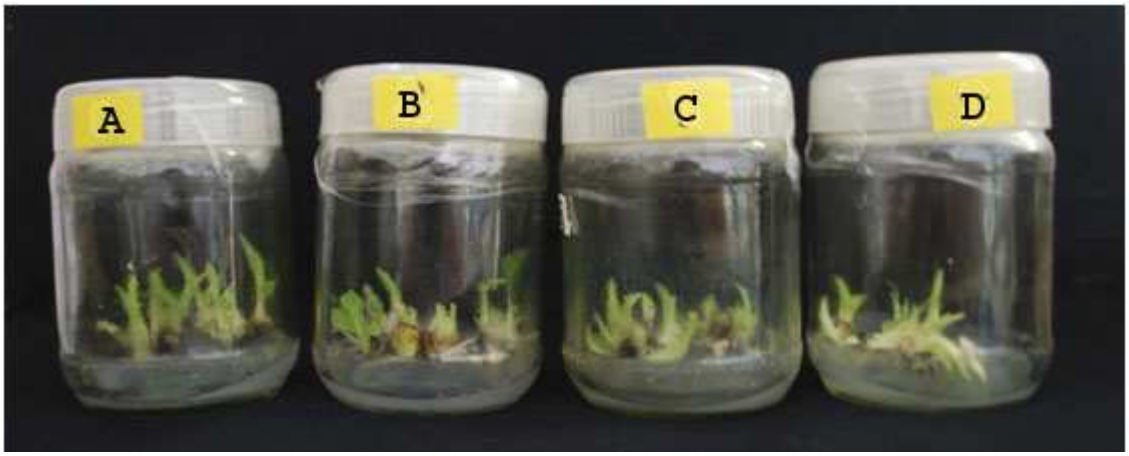

Figure 1: In Vitro Response of Multiplying Cultures of Banana Variety Robusta to Different Cytokinins by 15 Days of Culture. A. BAP 5 mg/L; B. 2-iP 5 mg/L; C. Kinetin 5 mg/L; D. Zeatin 5 mg/L

The multiplying cultures of *Robusta* containing 5 mg/L Kinetin had a multiplication ratio of 2.5. The cultures had moderate multiplication ratio in comparison to other cytokinins (BAP, 2iP, & zeatin) with moderate roots. It is observed that Kinetin can induce enhanced multiplication with profuse roots, but the quality of cultures was not satisfactory (Figure 10A-3). The results indicate that Kinetin may be suitable but may have to be supplemented with other cytokinin or auxin in smaller quantity in order to enhance the quality of cultures and also the multiplication ratio.

The multiplying cultures of Banana var. *Robusta* in media containing 5 mg/LZeatin showed multiplication ratio of 2.6 to 2.7 in 30th and 60th day old cultures respectively. The cultures had moderate multiplication ratio in comparison to other

Figure 2: In Vitro Response of Multiplying Cultures of Banana Variety Robusta to Different Cytokines after 30 Days of Culture. A. BAP 5 mg/L; B. 2-iP 5 mg/L; C. Kinetin 5 mg/L; D. Zeatin 5 mg/L; D. BAP 25 mg/L

Cytokinins (BAP, 2iP, kinetin) with less roots. Morphologically, the quality of cultures was comparatively inferior to other cytokinins (Figure 10 A-4). It is evident that use of zeatin alone in the medium may not be a suitable cytokinin for commercial multiplication of quality cultures. Moreover, zeatin is an expensive cytokinin to commercially exploit for routine tissue culture production.

The multiplying cultures of Banana cultivar *Robusta* in excess BAP media showed multiplication ratio of three. The shoot length was about 1.5 cm; shoot girth was about 1.4 cm; leaf breadth was about 2 cm with 2 to 3 leaves and two roots among 30^{th} and 60^{th} day old cultures. The cultures had highest multiplication ratio in comparison to cytokinins at the concentration of 5 mg/L (BAP, 2iP, kinetin & zeatin) with less roots. With second subculture i.e. at 60^{th} day, the quality of cultures had declined. Cultures became dwarf, pale in colour, hard with a large number of globular mass and shoots were inseparable. Although excess BAP enhanced multiplication, quality of cultures deteriorated with prolonged period of culture. This clearly indicates that, although BAP is an effective cytokinin, it is not recommended to be used at high concentration.

Intensive commercial micropropagation requires the optimization of not only the inorganic elements but also the other factors of the medium. This is systematically done by testing a range of concentrations of individual elements combined in various ways with other elements. Suggested methods have been a broad-spectrum approach using all possible combinations or a factorial approach where a range of each ingredient is tested, while other was held constant in consecutive subcultures (Anderson, 1980; Stimart, 1986). In the current investigation, a factorial approach was used wherein for all trials, different concentrations of BAP for multiplication and Labland's standard multiplication media was used as variable factors. At the same time, the constant factors included MS basal medium, 3 % sucrose and 8 % Agar.

It is evident from the above results that the multiplication of each of the explants inoculated into the media containing BAP has resulted in axillary shoot proliferation. This method of multiplication is the commercial technique adopted for clonal multiplication of most commercial crops. Axillary shoot proliferation was successfully employed for Banana cult. *Robusta* in this investigation. In this method, elongation of the terminal shoot is suppressed by trimming the shoot tip thereby promoting the proliferation of the axillary shoots. The process of axillary proliferation in *Robusta* is further augmented by the use of the BAP. The number of such clusters of microshoots developed per

explant in each culture cycle is known as multiplication ratio. The multiplication ratio of Banana cv. *Robusta* is strongly supported by BAP. It is obvious from the results that *Robusta* needs an optimum concentration of 5 mg/L for an ideal multiplication ratio. According to several published reports (Rahaman et al., 2004; 2013; Reddy et al., 2014) 4 mg/L BAP was optimum for adventitious bud differentiation for Banana cv. G9, Amritsagar, Agnishwar. Increased levels of cytokinin inhibit apical dominance and promote lateral shoot proliferation. This principle holds good for Banana cultivar *Robusta* without any multiplication ratio at zero BAP and higher multi ratio at 5 mg/L BAP. However, higher than 5 mg/L BAP was non-supportive for the normal growth of *Robusta* cultures. Similar results have been reported for Almond-Peach hybrid where optimum concentration of BAP for multiplication is 1 mg/L BAP and at 5 mg/L normal growth is affected (Hartmann et al., 1993).Cytokinins are known to reduce the dominance of apical meristems and induce axillary as well as adventitious shoot formation from meristematic explants (Pandey and Jaiswal, 2002). Amongst the cytokinins, BAP is the widely used, most effective and affordable cytokinin for the proliferation of multiple shoots (Johnson and Manickam, 2003). In the present study, triploid AAA cultivar produced maximum shoot multiplication in MS medium supplemented with 5 mg/L BAP and in the Multi-2 media of Labland.Each of the hormones tended to have an optimum concentration to achieve maximal shooting response. The better performance of 5 mg BAP compared to 2-iP, Kinetin and Zeatin could be due to metabolic inability. Similarly, concentrations of BAP at 25 mg/L BAP inversely affected the shoot multiplication. Multiple shoot production from MS medium supplemented with BA and IAA in lower concentration was reported in two diploid cultivars of South India (Mukunthakumar and Seeni, 2005). BAP at 5 mg/L was considered optimal for shoot proliferation as well as shoot elongation from excised scalps of banana cultivars (Shirani et al., 2011). Cronauer and Krikorian (1985a,b) obtained multiple shoot clusters from the terminal floral apices of Musa acuminata cv. Dwarf Cavendish (AAA), inoculated on modified MS medium supplemented with 5 mg/L BAP and 10 % (v/v) coconut water.

The high performance of BAP over the other cytokinins in inducing multiplication in shoot tip cultures has been reported in different cultivars of banana (Ikram-ul-Haq and Dahot, 2007a,b). The marked effects of BAP on shoot formation compared to kinetin and 2-iP as observed in this study may be attributed to its high stability in *in vitro* cultures which is in agreement with Buah et al. (2010). BAP is not easily broken down and therefore persists in the medium. It is also possible that the amount of BAP get conjugated in the medium was smaller than what happened to the other plant hormones. Therefore, larger amount of BAP existing in free or ionized forms in the medium are readily available to plant tissues.

CONCLUSIONS

The present investigation was undertaken to find out the efficacy of cost effective cytokinins such as BAP, 2-Ip, kinetin and zeatin for the production of quality banana plants. Among them, BAP was most effective in multiplication of banana. BAP at 5 mg/L in MS basal medium produced higher number of shoots and good quality of plants in Banana cultivar *Robusta* when compared to the other cytokinins.

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